Investigations on Dextran and its Application as a Plasma Substitute

BY

BJÖRN INGELMAN

Reprinted from Upsala Läkareförenings förhandlingar
Vol. 54 p. 107 (1949)

UPPSALA 1949
ALMQVIST & WIKSELLS BOKTRYCKERI AB

Investigations on Dextran and its Application as a Plasma Substitute

INAUGURAL DISSERTATION

By

BJÖRN INGELMAN

FIL. LIC., UPPL.

By due permission of the Philosophical Faculty of the University of Uppsala, to be publicly discussed in the lecture-room of the Institute of Chemistry, May 7th, 1949, at 10:00 A. M., for the degree of Doctor of Philosophy. In addition to this survey the papers numbered 6—15 in the references will be publicly discussed.

UPPSALA 1949

ALMQVIST & WIKSELLS BOKTRYCKERI AB

300/150

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41550014_0030

Reprinted from Upsala Läkareförenings Förhandlingar
Vol. 54 p. 107 (1949)

From The Institutes of Biochemistry and Physical Chemistry,
University of Uppsala, Sweden.

R. UNIV.
BIBLIOTHEEK
LEIDEN

Investigations on Dextran and its Application as a Plasma Substitute.

By

Björn Ingelman.

Introduction.

In 1941 the author started investigations on the colloids of the sugar beet under the guidance of A. Tiselius and in close collaboration with the Swedish Sugar Corporation. In 1942 while this work was in progress it happened that some of our sugar beet juices became infected with the bacterium *Leuconostoc mesenteroides*. From these infected juices the polysaccharide dextran, which was earlier described by sugar chemists, was isolated. Among the investigations which were performed at that time some attempts to immunize rabbits with this dextran should be mentioned. These experiments were performed in order to obtain an antiserum with the help of which it would perhaps be possible to show the occurrence of small quantities of dextran in sugar beet juices. It was found, however, that dextran, if very carefully purified, had no antigenic properties. This was one of the reasons why dextran was tried as a plasma substitute.

In January 1943 the author together with A. Grönwall started experiments in order to produce a plasma substitute from dextran and since then the main interest in dextran has been its medical use. During the war when these investigations started, there was an acute need for suitable solutions for the treatment of shock. The need of infusion solutions for this purpose is evident when one considers the extensive investigations on plasma and plasma fractionation which were carried out during the second world war especially in the U.S.A. It was also during wartime that interest in finding a substitute for blood and plasma for transfusion purposes was especially great. Even

during the first world war W. M. Bayliss tried to use solutions of gum-arabic as a plasma substitute. Infusion solutions containing gum-arabic were used for a while, but fell into disuse because of the occurrence of untoward reactions and as it was found that the substance was stored in the body (for example in the liver). The interest in plasma substitutes was also great in the fighting countries during the second world war and several substances of high molecular weight were tried in infusion solutions. However, no one of the tested preparations seemed to fulfill all the conditions required of a plasma substitute. The investigations on dextran gradually led to the development of what appears to be a good plasma substitute. This was made possible by the excellent collaboration of the scientific institutions in Uppsala, the physicians who performed the clinical tests, the pharmaceutical company Pharmacia and the Swedish Sugar Corporation. Unfortunately, dextran was not available for common use during the war as it had not yet emerged from the experimental stage. Technical difficulties prolonged the experiments and of course the clinical tests of a new infusion solution must be carried out with the greatest caution and necessarily take a long time. However, the plasma substitute produced from dextran, has now found extensive use in the treatment of shock and in shock prophylaxis in the civilian hospitals.

Before passing on to a survey of these dextran investigations it seems advisable to discuss briefly why plasma substitutes are needed, and what requirements a colloid should fulfill, in order to be qualified for use as a plasma substitute.

Blood, plasma and serum are used as therapeutic agents for the treatment of shock, e.g. after severe hemorrhage, contusions and extensive burns, as well as for shock prophylaxis in operations. The use of blood and plasma for the purpose of infusion is, however, accompanied by certain disadvantages. In order to obtain sufficient blood and plasma, a large and complicated organization with Blood Donor Centers is needed. It is troublesome to store whole blood or to separate the plasma, and of course blood and plasma are expensive. In the event of war or other catastrophies it is quite impossible to meet in full the demand for blood and plasma. In peacetime it is also extremely difficult to obtain sufficient quantities of blood and plasma. Blood and plasma are very delicate solutions for infusion; they cannot undergo sterilization by heating. Therefore there is always a definite risk that certain infectious diseases will be transmitted by the infusion. At times both blood and plasma infusions give rise to undesirable reactions. Furthermore with blood infusions special attention must be paid to the blood groups and compatability of the patients.

It is therefore only natural that there have been a number of investigations in order to find solutions which could replace the expensive and delicate blood or plasma for the treatment of shock. Sub-

stances of high molecular weight such as gum-arabic, gelatin, pectin, polyvinylalcohol, polyvinylpyrrolidon, plasma of animals etc. have been tested for this purpose. The infusion of these colloids has however been accompanied by certain disadvantages. Some of the substances tested have given undesirable reactions, some have antigenic properties, and some could not be broken down by the organism, hence they accumulated e.g. in the liver.

For the treatment of shock caused not only by hemorrhage but also by contusions and burns and in shock prophylaxis, it is necessary by means of the infusion of suitable solutions to increase the volume of the circulating blood, so that the blood pressure may be maintained at a normal value. This cannot be done in a satisfactory way with crystalloid solutions, since substances of low molecular weight quickly leave the blood-stream. Instead infusion solutions are required containing colloids which produce the same colloidal-osmotic pressure as the plasma proteins. In order for the colloids to produce this pressure and keep the water in the blood vessels, they should have such a large molecular size that they do not pass out through the walls of the blood vessels but remain in the blood-stream. The molecular weight of the colloid should consequently not be too small. On the other hand it should not be too large as the colloidal-osmotic pressure then becomes too small and the risk of undesirable reactions seems to increase with the increasing molecular weight of the colloid. It has been found that the molecular weight should be of the order of magnitude of 100 000. (Of course it is rather the dimensions etc. than the weight of the molecules which are of importance in this connection.) It is not necessary for the substance to be monodisperse. However, it probably is desirable that there should be a suitable distribution of the molecular weights. The solution of the plasma substitute must also contain crystalloid substances (preferably NaCl) so that it may be isotonic with blood.

Solutions of plasma substitutes as well as plasma solutions do not contain blood corpuscles. As, however, a resting person can get along with a considerably reduced number of erythrocytes, it is often unnecessary for the infusion solution to contain them. In certain types of shock, involving an increased concentration of blood corpuscles, erythrocytes must be absent from the infusion solution. If, however, the patient has a very low blood count whole blood must be given.

It must be possible to infuse the plasma substitute intravenously, in large quantities, and on repeated occasions without incurring undesirable reactions. It must consequently be quite non-toxic and possess no antigenic properties. One should of course be able to administer the substance to all, irrespective of blood-groups and the like.

The solutions must not be too viscous. Preferably the viscosity should be of the same order of magnitude as that of plasma (even at room temperature).

The substance should be of such a nature that the body is able to eliminate it at a suitable rate, so that it does not remain in the blood for too short or too long a period or accumulate in the organs.

It should be possible to sterilize the colloid solution by means of heating to insure the fact that neither bacteria nor virus infections are transmitted. The sterilized solutions should be very stable so that the flasks containing the prepared solutions can be kept for a long time at room temperature without special precautions. From the standpoint of military medicine it would be highly desirable to have a solution which could tolerate freezing — such as might occur in a winter war — and subsequent heating for use.

Finally the substance should lend itself to large scale production, so that it could be made available at a far lower cost than plasma and plasma fractions.

It is not so difficult to find colloids which only partially fulfill the conditions set forth, but earlier work has shown that it is very difficult to prepare a colloid which simultaneously fulfills all these conditions.

It has now been shown that by means of the partial breakdown of the polysaccharide dextran a substance can be prepared which fulfills the requirements indicated above for a plasma substitute.

Chemical investigations.

Dextran was discovered during the middle of the last century by sugar chemists. It was found that this polysaccharide was formed in sugar beet juices which were infected with the bacterium *Leuconostoc mesenteroides* or similar micro-organisms. Earlier, infections of this bacterium caused a great deal of trouble in the sugar industries on account of sugar losses and because juices containing dextran filtered slowly. (On the other hand, smaller amounts of dextran in the sugar juices do not decrease the velocity of crystallization of saccharose (1, 2, 3).) During the last century it was shown that dextran on total hydrolysis yields glucose. Later, investigations on the structure of dextran indicate that the dextran molecule is built up of a main chain of glucose units. To this main chain, which is thought to be of considerable length, shorter side chains of glucose units are attached. Evidence for this structure has been obtained by hydrolysis of methylated dextran and identification of the degradation products. Most of the glucopyranose units in the dextran molecule are joined by 1,6-D-glucosidic bonds, but 1,4-D-glucosidic bonds can also occur. It is assumed that these bonds have an α-configuration. (For earlier chemical and bacteriological work on dextran see the references given in

our papers, and in T. Evans' and H. Hibbert's survey of bacterial polysaccharides (4).)

The main purpose of these dextran investigations has been the preparation of a plasma substitute. While this work was in progress it was found more and more necessary to acquire additional knowledge of the properties of this polysaccharide that was not to be found in the literature. However, a closer study of this substance is also interesting from other points of view. It may for instance be mentioned that dextran is one of the few polysaccharides which hitherto has been synthesized from low molecular substances with the aid of cellfree enzyme extracts and that, in contrast to the synthesis of starch and glycogen, glucose-1-phosphate is not an intermediate product in the enzymatic synthesis of dextran. It has been shown that enzyme extracts obtained from cultures of *Leuconostoc mesenteroides* convert saccharose to dextran and fructose. (Cf. e.g. E. Hehre's articles (5).)

In order to establish the fact that the polysaccharide obtained from the bacterial cultures used here was dextran, and in order to further characterize the substance, various analyses were performed (6). The substance which had been purified by means of precipitation with alcohol and dialysis in cellophane bags, had a specific rotation $[\alpha]_D^{20^\circ}$ of $+195^\circ$ in aqueous solution. It was also shown that the isolated polysaccharide yielded glucose on total hydrolysis. Electrophoresis experiments in the Tiselius Apparatus showed that the polysaccharide was an uncharged substance. The polysaccharide was investigated in Svedberg's ultracentrifuge. It was found that the sedimentation constant was highly dependent on the dextran concentration of the solution. At low concentrations the sedimentation constants were very high. ($s \approx 36$ *S* at 0.4 per cent.) The diffusion constant was also determined and found to be low. ($D_A \approx 0.3 \cdot 10^{-7}$ at 0.35 per cent.) From the sedimentation and diffusion constants it could be calculated that the molecular weight of dextran was extremely high, in the order of magnitude of several millions. An exact value for the molecular weight could not be given for this unfractionated dextran, as the substance was too polymolecular. The high viscosity of the aqueous solutions of dextran, the very marked concentration dependence of the sedimentation constants found in the ultracentrifuge experiments, and other measurements indicated that dextran molecules are more or less threadlike (6).

Investigations of this very high molecular polysaccharide in the electron microscope, performed together with K. Siegbahn in 1943, also showed that dextran has a threadlike structure. There are many factors indicating that the long branched threads, seen in the electron micrographs, are single gigantic dextran molecules. The thickness of these threads at the narrowest places which are visible, is 30—100 Å (7, 8).

By means of partial hydrolysis of the very high molecular dextran with the aid of acids it is possible to obtain products with molecular weights of the order of magnitude of 100 000. Partially hydrolyzed dextran with molecular weights in a suitable range (20 000—200 000) is well suited for use as a plasma substitute (9, 10, 11, 12, 13). In order to obtain such products it is of course possible to vary the three factors: temperature, time and acid concentration within quite wide intervals. Of course the time of hydrolysis is also dependent on the degree of polymerization of the very high molecular, raw dextran. Only one example of such a partial hydrolysis of dextran will be given here: the hydrolysis reaction was in this case carried out on a 8 per cent solution of a high molecular dextran. The temperature was 90° C. Hydrochloric acid was added until the solution became 0.12 *N*. After 20 minutes the hydrolysis was interrupted by neutralization with sodium hydroxide. After hydrolytic treatment the dextran was precipitated with alcohol and dried. A 6 per cent solution of this partially hydrolyzed dextran was prepared, the relative viscosity of which was 3.3. A neutral solution of such a partially hydrolyzed dextran can be sterilized by heating at 120° C for 30 minutes. This treatment decreases the viscosity only very slightly.

Dextran and its partially degraded products are polymolecular, i.e. consist of molecules with the same chemical composition but with different molecular weights. Therefore, in the formula $(C_6H_{10}O_5)n$, the number n can vary within a rather large range for the molecules of a dextran preparation. In order to investigate the possibility of obtaining dextran preparations, more homogeneous in respect to the size of the molecules, fractionation experiments were carried out. As will be described later in this survey, the usefulness of dextran as a plasma substitute is highly dependent upon the size of the dextran molecules. Unhydrolyzed or only slightly hydrolyzed dextran infused intravenously will cause injurious reactions. However, dextran, broken down to a molecular size of the same order of magnitude as that of the plasma proteins, is useful for the infusion solutions. In producing a plasma substitute from dextran it is necessary to interrupt the hydrolysis at a stage when a major portion of the dextran molecules have such a large molecular size that they stay for some time in the blood-vessels and are not immediately filtered out through the glomerular membranes of the kidneys or through the walls of the blood-capillaries. On the other hand, the molecular weight has to be low enough to give a high colloidal-osmotic pressure. By performing a fractionation procedure on inhomogeneous, partially hydrolyzed dextran it is possible to produce a more homogeneous preparation. As the largest and the smallest molecules are removed from this product, it is more suited for therapeutical purposes. By fractionation experiments it is also possible to be certain that different batches of the plasma substitute have

suitable distributions of molecular weights. Furthermore, it is also of great interest to get more homogeneous dextran fractions for physico-chemical investigations.

It has been shown that dextran preparations of different degrees of degradation can be divided up into a number of more homogeneous fractions by means of fractional precipitation with e.g. ethyl alcohol. The author and M. S. HALLING have published an article describing some of these fractionation experiments (14). In connection with these experiments physico-chemical investigations were made on some of the dextran fractions. As a starting material for these fractionation experiments dextran preparations of highly different degrees of degradation were used. Therefore, the fractions obtained covered a very large molecular weight interval. Most of the fractions were characterized by means of viscosity measurements at different concentrations, and by determinations of intrinsic viscosity. Several fractions were investigated in SVEDBERG's ultracentrifuge and in CLAESSON's diffusion cell at different concentrations. The sedimentation and diffusion constants of the fractions of the highest molecular weights showed a marked concentration dependence. With the aid of the sedimentation and diffusion constants, extrapolated to zero concentration, the molecular weights and the shape of the molecules were calculated. For the fraction of the highest molecular weight investigated, a molecular weight of about 40 000 000 was calculated, and the lowest molecular weight was found to be 14 000. The experiments indicated that the dextran molecules are more or less thread-like. The fractions of the highest molecular weights have large molecular lengths. The relation between intrinsic viscosity and molecular weight of the fractions was given in diagrams. Compared to molecular weight determinations by means of ultracentrifugation and diffusion, viscosity measurements are rather easy to perform. Therefore, such diagrams are of great value both in the manufacture of dextran and in physiological and clinical investigations. From the diagrams an estimation of the molecular weight of a dextran fraction obtained can be made from viscosity determinations.

Two fractions belonging to the molecular weight interval (20 000—200 000), which is of special importance in the preparation of the plasma substitute had intrinsic viscosity values of 0.25 and 0.29. The corresponding molecular weights were 84 000 and 141 000. The thickness of the molecules of these fractions was of the order of magnitude of 20 Å (14).

With respect to the preparation of the plasma substitute it is of particularly great interest to know the size of the molecules in the fraction of the highest molecular weight which passes through the kidney membranes. Therefore, in connection with the investigations described above, dextran, isolated from urine of patients given intra-

venous infusions of partially hydrolyzed dextran, was fractionated into four fractions. The molecular weight calculated for the fraction of the highest molecular weight was 36 000. The intrinsic viscosity was 0.18. Of course the fraction was not quite homogeneous. Therefore, molecules somewhat larger in size than this average value indicates, will pass from the blood-stream through the glomerular membranes of the kidney out in the urine (14).

In connection with the fractionation experiments the adsorption on active carbon was investigated for some of the fractions. It was found that the adsorption increases as the molecular weight of the dextran fractions decreases. However, in the series glucose, disaccharide, trisaccharide etc., the adsorption increases with increasing molecular weight. Therefore the adsorption passes through a maximum when the molecular weight increases successively from that of glucose, disaccharide, trisaccharide etc. to that of more and more high molecular dextrans (14).

Some of the fractions of very high molecular weights were also investigated in the electron microscope using the gold shadow casting method. The electron micrographs obtained of the fractions of the highest molecular weights showed long, branched threads similar in appearance to those obtained in the earlier electron microscope investigations (14).

The possibilities of performing the partial hydrolysis of dextran with the aid of enzymes has also been investigated. Several years ago the author found that it was possible to cultivate the bacterium *Cellvibrio fulva* on dextran solutions and that this bacterium breaks down dextran. From this micro-organism, grown on dextran solutions, it was possible to prepare active enzyme extracts. The enzymatic breakdown of dextran was studied particularly by means of viscosity measurements. The experiments indicated that during the first stage of the breakdown this enzyme chiefly splits the dextran into comparatively large fragments (15). The discovery of enzymes which break down dextran is also interesting from a biochemical point of view because of the occurrence of 1,6-D-glucosidic bonds in dextran. Recently L. Nordström and E. Hultin have also found that it is possible to cultivate certain moulds on dextran solutions and to prepare dextran splitting enzyme solutions from such cultures (16).

In order to test the dextran—obtained with the bacterial cultures used here—for antigenic properties, rabbits were injected intravenously several times over a period of weeks with dextran. No precipitin reactions occurred when dextran solutions were added to the sera in different dilutions. As has been reported by other investigators, carefully purified dextran is not antigenic.

It has been reported in the literature that dextran (not partially hydrolyzed dextran) gives precipitin reactions not only with anti-

Leuconostoc sera but also with pneumococcus Types II, XII and XX antisera. (Cf. e.g. the survey given by T. Evans and H. Hibbert (4).) In an unpublished investigation, performed by the author, it was shown that the partially hydrolyzed dextran of the type used as a plasma substitute did not give precipitin reactions with pneumococcus Types II, XII and XX antisera.

Some dextran derivatives have also been produced. Among these a dextran sulphuric acid ester with high anticoagulant activity may be mentioned (17).

Physiological investigations.

In experiments with rabbits it was rather quickly shown that the viscous aqueous solutions which are obtained from the natural dextran could not be injected intravenously. Such solutions of very high molecular dextran caused severe injury to the experimental animals. But when the dextran was partially hydrolyzed with acids to a molecular weight of the same order of magnitude as that of the proteins of blood and the product carefully purified, the test animals could be injected with large quantities without any injurious results (9, 10, 11, 12, 13). The relative viscosity of 6 per cent solutions of partially hydrolyzed dextran used for the animal experiments was between 10 and 3. During the physiological and clinical experiments the relative viscosity was decreased so that the viscosity of the 6 per cent solutions now used as plasma substitute is between 3 and 4. The solutions also contained a suitable amount of NaCl and the solutions were sterilized by heating.

An investigation was made of how the dextran concentration in experimental animals' blood decreased with time after infusion, and it was found that the period of time during which dextran stays in the blood is rather ideal. After intravenous infusions of therapeutic doses of partially hydrolyzed dextran the concentration of the plasma substitute in the blood sinks to 0 in about 5 days. It was also shown that a small part of the polymolecular dextran, namely the fraction which has the smallest molecular size, is filtered out through the kidneys into the urine (10).

Even after repeated large intravenous infusions no injurious reactions followed. Some time after the last injection the animals were killed and examined histologically by N. Gellerstedt, without any sign of injury or accumulation being observable (10). One dog has now been given about 100 infusions of dextran for the past 4 years without injurious effect. (About 150—200 ml of a 6 per cent sterile solution were administered each time. The solution also contained 0.9 per cent NaCl. The amount of dextran solution administered cor-

responds to more than the body weight (about 15 kg) of the dog.) Rabbits were also injected with large quantities of partially hydrolyzed dextran for long periods of time. The amount of dry dextran given corresponded to about 2 per cent of the body weight. These animals were killed some months after the last injection. Liver, kidneys, spleen, and muscle were ground and extracted: partly with water, partly with NaOH solution, after which the extracts were tested for dextran content. In earlier experiments dextran determinations were performed with the aid of rotation determinations. (Before the rotation determinations proteins were removed with trichloracetic acid, and glycogen was removed with amylase, which does not break down dextran. After this the solutions were dialyzed.) Later, HINT and THORSÉN's method for the determination of dextran was used (18). The method is sufficiently sensitive to allow for the detection of dextran in small quantities even in the presence of glycogen. No dextran could be detected in the extracts within the limits of error for the methods. (Less than 0.2 per cent dextran in the organs.) Since large quantities of dextran were injected, dextran must have been slowly broken down in the body. (These animal experiments are not completely described in our earlier papers. It is, however, no longer important to prepare a complete review of these early animal experiments as so many clinical results are now available. In connection with the clinical investigations extensive animal experiments have also been performed.) Further support for this indirect proof that dextran is broken down in the body is provided by certain hormone investigations which the author has performed. If dextran-injected rabbits are given thyroid gland extract, the dextran disappears from the blood more quickly than usual. The most probable interpretation of this result is that when the metabolism is increased by means of the thyroid gland hormone, the dextran is broken down more quickly. (Unpublished investigations (13).)

In order to further establish dextran's innocuous nature, the author made an investigation which showed that dextran injections do not influence the growth of young animals. (The experiment was performed several years ago but has not been previously published.) If young growing animals are exposed to some harmful influence, the effects are often seen on their growth curves. Seven young rabbits of approximately the same size and from the same litter were used. During a period of 50 days the animals were weighed regularly. About every five days three of the animals were subjected to intravenous infusion of 10 ml dextran solution each. (Partially hydrolyzed dextran of the type used as a plasma substitute was administered.) All animals were subjected to the same living conditions throughout the course of the experiment. The growth curves for the dextran treated rabbits were the same as those for the control animals. (Before the experiment the weights of the control animals were 0.70, 0.75, 0.80 and 0.85 kg and

after the experiment 1.80, 1.80, 1.90 and 2.00 kg respectively. The corresponding values for the dextran treated rabbits were 0.75, 0.80, and 0.80 kg before and 1.90, 1.90 and 1.95 kg after the experiment.) Judging from this single experiment, dextran infusions do not influence the growth curves of young rabbits.

It was found that after dextran infusion the sedimentation rate of the erythrocytes is increased. The dextran of very high molecular weight produces a greater sedimentation than the partially hydrolyzed dextran. The sedimentation rate of the erythrocytes decreases the more the dextran is broken down (10). A similar increase in the sedimentation rate has been reported after infusion of other tested plasma substitutes. The pseudo-agglutination of the red blood corpuscles and the increase in the sedimentation rate after infusion of therapeutic doses of partially hydrolyzed dextran do not seem to have an injurious influence. In unpublished investigations by Hint and Thorsén it has been shown how the sedimentation rate of the erythrocytes increases with increasing molecular weight of dextran. For these experiments dextran fractions were used which were more homogeneous in respect to molecular weight than the unfractionated partially hydrolyzed dextran.

Determinations of the colloidal-osmotic pressure of solutions of partially hydrolyzed dextran gave values of the same order of magnitude as those for plasma solutions of the same concentrations. As the animal experiments have shown that dextran remains in the bloodstream for a sufficiently long time, one would expect that infusions of partially hydrolyzed dextran should give a sufficient contribution to the colloidal-osmotic pressure of the blood to guarantee a beneficial effect in treatment of shock. The animal experiments also showed that this was the case. The therapeutic effect was studied on experimental animals in a state of shock, induced by bleeding, histamine and contusion which were produced in anesthetized rabbits and cats. Good effects on blood pressure, heart activity and respiratory rate could always be recorded (10, 13).

In view of the fact that the animal tests were successful and promising, clinical tests were begun on a very limited scale. Since these tests gave promising results, extensive clinical investigations were begun by G. Bohmansson and O. Wilander. Later, dextran was also subjected to tests by G. Thorsén, H. Rosenqvist and other surgeons and physiologists. However, before passing on to a very brief review of the clinical tests, an overall view will be given of the arrangements made for the large scale production of this plasma substitute. (In connection with clinical tests it must always be understood that the dextran referred to is the partially hydrolyzed product of the type used as a plasma substitute.)

Large scale production of the plasma substitute.

Raw dextran is now produced by the Swedish Sugar Corporation. The cultivation of *Leuconostoc mesenteroides* is performed in sterile sugar solutions under well standardized conditions. The same bacterial culture is used throughout. The very high molecular dextran, formed in the sugar solution, is precipitated with alcohol. Then, in AB. Pharmacia, the dextran is partially hydrolyzed with the aid of HCl. After this the dextran is purified by several reprecipitations and filtrations through adsorbents after which the material is dried *in vacuo*. The final dried product can be stored in closed containers for an apparently unlimited time. The specific rotation $[\alpha]_D^{20^\circ}$ of this material is + 194°. (This is an average value of determinations on six different substances. The determinations were performed with solutions containing about 1 per cent dextran.) Solutions containing 6 per cent partially hydrolyzed dextran and 0.9 per cent NaCl are prepared and then sterilized at 110° C. At present bottles containing 380 or 800 ml of dextran solution are used. The relative viscosity of the 6 per cent solutions is between 3 and 4. (During the clinical tests it was found advisable to decrease somewhat the viscosity and the molecular weight. Thus the viscosity is now lower and the colloidal-osmotic pressure higher than in the material used for the initial physiological and clinical investigations.) The solutions are subjected to bacteriological, physiological and chemical controls. It is of utmost importance that the substance is of high purity. (The solutions must be so pure that one can, for example, after severe burns when large losses of plasma take place, inject up to ten liters intravenously over a period of some days into one and the same patient.) The flasks containing the sterile solutions can be stored at room temperature for a long time without special precautions.

During the physiological tests it was shown that the solutions should not contain dextran molecules of too large a size. Each batch of the plasma substitute is therefore controlled so that it does not contain fractions of dextran which have too high a molecular weight. The question concerning the most suitable distribution of molecular weights has been paid the utmost attention, and methods have been worked out for the fractionation of the polymolecular dextran into products more homogeneous with respect to molecular weight.

Clinical tests and further physiological investigations.

Since the comprehensive clinical tests of partially hydrolyzed dextran were begun by G. Bohmansson and coworkers some years ago, about 40 000 bottles of dextran have been used in hospitals up to Feb-

ruary 1949. Therefore, the results of extensive experience with this plasma substitute have been collected (19—35).

The clinical tests have shown that the result of shock treatment with dextran is good and that when required several liters of dextran solution can be injected without the occurrence of injurious secondary reactions. The dextran concentration in plasma can rise to several per cent, in which case the concentration of protein can be appreciably lower than normal. The concentration of the colloids seems to adjust itself so that the colloidal-osmotic pressure of the blood becomes normal. A part of the blood-protein can consequently be temporarily replaced by dextran without inconvenience. Afterwards, new plasma protein is formed at the same time that dextran disappears from the blood.

An initial report on the clinical results of dextran treatment was presented before the Swedish Association of Surgeons in 1945 by G. Bohmansson, H. Rosenqvist, G. Thorsén and O. Wilander (19). The results were promising and they made the statement that dextran is a good therapeutic agent in shock of various origins and is also of great value as a shock prophylactic. Mention of the fact can be made that they found that the bleeding and coagulation times were not affected after administration of therapeutic doses of dextran. Neither did they find any injurious effects on liver or kidney functions. These preliminary results, which were presented at that time, have been confirmed by later clinical investigations. Later, in his thesis on the treatment of extensive burns, H. Rosenqvist judges the effect of dextran to be as valuable as that of plasma (20).

At the Scandinavian Surgical Congress in June 1947 the clinical results of dextran infusions on over 800 patients were presented by G. Bohmansson and G. Thorsén (21, 22). All patients were thoroughly studied. None of the cases which has come to post-mortem examination have shown any tissue damage that might arouse suspicion of injurious effects or of the accumulation of dextran. Thorsén maintained that after operations latent shock is present in so many cases that more attention should be devoted to shock prophylaxis than is often the case. For the purpose of shock prophylaxis colloidal and not crystalloidal solutions should be infused. This implies that dextran can be used not only for accidents and extensive burns or operations where shock has already been sustained, but also in the daily routine of surgical departments to prevent shock in operations. Bohmansson summed up the results of the clinical tests with the statement that dextran is a safe and very good plasma substitute for the treatment of shock and for the prevention of shock. At the same congress W. Kock reported good results for the intravenous administration of dextran in pediatric cases (23).

After June 1947 dextran was released for general medical use.

T. Hiertonn has described good results with dextran in appendical peritonites (24).

Over a period when about 16 000 bottles of dextran containing 380 ml each were used, very few cases of unfavourable reactions were reported. Untoward reactions of the same type have been encountered with much greater frequency after transfusions of blood, plasma, and other plasma substitutes (25). In the same article G. Thorsén describes animal experiments in which extremely large quantities of dextran were administered over a period of several days. (After infusions, corresponding to therapeutic doses no injurious effects were noted even after a week long infusion series. After infusions over a period of several days, with quantities far higher than the therapeutic doses, the animals became sluggish and edematous. The largest quantities given would, for a fully grown person, correspond to ten liters per day for several days. Shortly after the termination of the infusion series, the animals quickly recovered. With dextran therapy just as for all other types of liquid therapy, and for the administration of all drugs, the dosage should be carefully selected to fulfill the patient's need.) For the present Thorsén's experiments suggest that wound healing is not impeded by the administration of therapeutic doses of dextran with the resulting dextran concentrations of 1—2 per cent in the plasma. G. Thorsén and N. O. Heinertz have begun experiments to ascertain whether the resistance to infection and the formation of antibodies are to any extent altered after the administration of dextran.

M. Goldenberg, R. Crane and H. Popper have described the effect of intravenous administration of dextran into dogs, rabbits and guinea pigs (26). Even the intravenous administration of high amounts of dextran did not interfere with the health and well-being of the experimental animals. (The largest amount given was more than 1 kg of the solid material to a dog weighing 24 kg.)

In a paper read to the Annual Meeting of the Association of Surgeons of Great Britain and Ireland in 1948, G. Thorsén gives a survey of the experiments performed on dextran in Sweden, where today in hospital preparedness for emergency cases, dextran is relied upon to a large extent for the transfusion service. In this paper diagrams are given of the variations of colloidal-osmotic pressure, plasma protein concentration and dextran concentration in plasma after repeated parenteral administration of dextran. H. Malmros and G. Thorsén are conducting experiments to determine the effect of dextran infusions on the albumin and globulin ratios of the plasma. In Thorsén's paper dextran is recommended as a substitute for plasma in cases of shock, for shock prophylaxis and for cases with hypoproteinemia when the effect of an increase in the colloidal-osmotic pressure is desired (27).

During the past few years dextran has been tested by J. Lundy at the Mayo Clinic, Rochester, Minn., U. S. A. The first dextran solu-

tions tested produced unfavourable results. (Raw material for these solutions was obtained from Sweden, but the preparation of the solutions was performed in the U. S. A.) However, good results were obtained from later tests. The following quotation is cited from J. LUNDY's most recent report: "Recently dextran, bottled in Stockholm (Pharmacia) and tested for particle size has been made available to us and there have been no untoward reactions to this material among either the anesthetized or unanesthetized individuals. It would appear from our experience that this material, when prepared in this way, is an excellent substitute for plasma and might well become more useful than plasma in supportive therapy."—"It seems desirable at least to one of us (J. S. L.) that this material should be manufactured in our own country and be made available for both civilian and military use and that this may be an answer to the editorial on the need for plasma substitute" (28).

K. BANG RASMUSSEN has published a report of the results obtained from the use of 125 portions of dextran at a hospital in Denmark. All infusions had good effects. Secondary effects were not observed (29).

At an international congress of military surgeons in 1948 a survey of the clinical use of dextran was given by G. BOHMANSSON and A. GRÖNWALL (30, 31).

In a recently published article J. P. BULL, C. RICKETTS, J. R. SQUIRE, W. MAYCOCK, S. J. L. SPOONER, P. L. MOLLISON and J. C. S. PATERSON describe investigations on dextran in England. As a means of detecting the presence of dextran in the tissues they made use of the fact that antisera to *Leuconostoc mesenteroides* give a precipitin reaction with dextran, which behaves as a haptene. They found that good antisera prepared in rabbits react with dextran in concentrations of 1 : 1 000 000. With this very sensitive method they studied how long a time traces of dextran could be detected in different organs. It was shown that the dextran could be detected for the greatest length of time after the infusion in the lymph-glands and the spleen. In animal experiments as well as in clinical tests they found that the dextran solution used was well tolerated, gave no unfavourable reactions and proved efficacious as a plasma substitute (32).

Acknowledgement.

I wish to express my sincere gratitude to my teacher Professor A. TISELIUS for his never failing interest in this work, for encouraging discussions and for all help in different ways.

I am very grateful to Professor T. SVEDBERG for placing the vast resources of the Institute of Physical Chemistry at my disposal.

To my friend Laborator A. GRÖNWALL, I desire to express my deep gratitude for excellent collaboration during the six years these investigations have been in progress.

I also wish to extend my thanks to the coworkers and the members of the staffs

of the Institutes of Biochemistry and Physical Chemistry as well as the scientists of other institutes in Uppsala who have aided in this work.

The clinical tests of dextran were performed under the guidance of Professor G. Bohmansson to whom I wish to express my gratitude. Also I wish to thank Drs. H. Rosenqvist, G. Thorsén, O. Wilander as well as Dr. H. Hint and other physicians who have taken part in the tests for their interest in this work. Without their valuable clinical investigations of dextran it would not have been possible to carry out the work which has led to the production of this plasma substitute.

Grateful acknowledgement is made to Mr. E. Göth, President of AB. Pharmacia and Mr. T. Wintzell, President of the Swedish Sugar Corporation for their support in these investigations. I should also like to thank all the coworkers of AB. Pharmacia and the Swedish Sugar Corporation who have taken part in the experiments for the large scale production of this plasma substitute.

References.

1. Ingelman, B. "The Svedberg 1884 30/8 1944", Uppsala (1944) 155.
2. —— Socker, *1* (1945) 179.
3. —— Socker, *3* (1947) 5.
4. Evans, T., and Hibbert, H. Advances in Carbohydrate Chem. *2* (1946) 203.
5. Hehre, E. Trans. N. Y. Acad. Sci. *10* (1948) 188.
6. Grönwall, A., and Ingelman, B. Acta Physiol. Scand. *7* (1944) 97.
7. Ingelman, B., and Siegbahn, K. Arkiv Kemi, Mineral. Geol. *18 B*, N:o 1 (1944).
8. —— Nature *154* (1944) 237.
9. Grönwall, A., and Ingelman, B. Nord. Med. *21* (1944) 247.
10. —— Acta Physiol. Scand. *9* (1945) 1.
11. —— Nature *155* (1945) 45.
12. Ingelman, B. Socker *1* (1945) 188.
13. —— Acta Chem. Scand. *1* (1947) 731.
14. Ingelman, B., and Halling, M. S. Arkiv Kemi *1* N:o 18 (1949).
15. Ingelman, B. Acta Chem. Scand. *2* (1948) 803.
16. Nordström, L. and Hultin, E. Svensk Kem. Tid. *60* (1948) 283.
17. Grönwall, A., Ingelman, B. and Mosimann, H. Upsala Läkareförenings Förhandlingar. Ny följd. Bd L (1945) 397.
18. Hint, H. C. and Thorsén, G. Acta Chem. Scand. *1* (1947) 808.
19. Bohmansson, G., et al. Acta Chir. Scand. *94* (1946) 149.
20. Rosenqvist, H. Acta Chir. Scand. *95* (1947) suppl. 124.
21. Bohmansson, G. Transactions at the twenty-third meeting of The Northern Surgical Association in Stockholm 1947, 129.
22. Thorsén, G., ibid., 126.
23. Kock, W., ibid., 132.
24. Hiertonn, T. Acta Chir. Scand. *96* (1947) 224.
25. Thorsén, G. Nord. Med. *40* (1948) 2374.
26. Goldenberg, M., Crane, D. and Popper, H. Am. J. Path (1947) 939.
27. Thorsén, G. Lancet *256* (1949) 132.
28. Lundy, J., et al. Proceedings of the Staff Meetings of the Mayo Clinic. *23* (1948) 444.
29. Bang Rasmussen, K. Nord. Med. *40* (1948) 2381.
30. Bohmansson, G. Bulletin International des Services de Santé des Armés. In press.
31. Grönwall, A. Bulletin International des Services de Santé des Armés. In press.
32. Bull, J. P., et al. Lancet *256* (1949) 134.
33. Bohmansson, G., et al. J. Internat. Chirurgie *8* (1948) 890.
34. Hiertonn, T. Svenska Läkartidningen *45* (1948) 1261.
35. Bohn, H. Svenska Läkartidningen *45* (1948) 2347.

Uppsala 1949. Almqvist & Wiksells Boktryckeri AB 48882